Claims

1. A circularly permuted avidin monomer, **characterized** in that the carboxyl terminal amino acid and the amino terminal amino acid of the polypeptide of the avidin monomer have been joined directly or via a linker, and new carboxyl and amino termini have been created by cleavage of the polypeptide and the resulting circularly permuted avidin monomer binds biotin or other ligand.

2. The circularly permuted avidin monomer of claim 1, **characterized** in that the avidin is selected from wild type avidin, mutant form of avidin, streptavidin and variant of avidin, such as other poultry avidins and chicken avidin-related proteins (AVRs).

3. The circularly permuted avidin monomer of claim 1, **characterized** in that the carboxyl terminal amino acid and amino terminal amino acid have been joined by a linker comprising one or more amino acids.

4. The circularly permuted avidin monomer of claim 3, **characterized** in that the linker is a hexapeptide comprising four glycine residues and two serine residues and wherein one glycine is connected to the carboxyl terminal amino acid and one serine is connected to the amino terminal amino acid.

5. The circularly permuted avidin monomer of claim 1, **characterized** in that the circularly permuted monomer is cpAvd5→4.

6. The circularly permuted avidin monomer of claim 1, **characterized** in that the circularly permuted monomer is cpAvd6→5.

7. The circularly permuted avidin monomer of claim 1, characterized in that the circularly permuted monomer is cpAvd4→3.

8. The circularly permuted avidin monomer of claim 5, 6 or 7, **characterized** in that the monomer has been mutated.

9. The circularly permuted avidin monomer of claim 5, 6 or 7, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to any other amino acid residue X and/or the isoleucine residue 117 to any other amino acid residue X and/or the serine residue 16 to any other amino acid residue X and/or the threonine residue 35 to any other amino acid residue X and/or the asparagine residue 118 to any other amino acid residue X, (Y33X, I117X, S16X, T35X, N118X).

10. The circularly permuted avidin monomer of claim 9, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to histidine residue and/or the isoleucine residue 117 to cysteine residue and/or the serine residue 16 to alanine residue and/or the threonine residue 35 to alanine residue and/or the asparagine residue 118 to methionine, (Y33H, I117C, S16A, T35A, N118M).

11. A dual-chain avidin (dcAvd), characterized in that it comprises a fusion of two of the monomers selected from the circularly permuted monomer cpAvd5→4, the circularly permuted monomer cpAvd6→5, and the circularly permuted monomer cpAvd4→3, or the mutated monomer forms of one of the claims 8 to 10, and the resulting dual-chain avidin binds biotin and/or other ligand.

12. A dual-chain avidin of claim 11, characterized in that the two monomers are fused together directly or joined by means of a spacer.

13. A dual-chain avidin of claim 12, characterized in that the spacer is a peptide spacer from about 1 to 40 amino acid residues.

14. A dual-chain avidin of claim 13, characterized in that the spacer is a peptide SGG or SGGS.

15. The circularly permuted avidin monomer of claim 1, **characterized** in that the biotin-binding affinity of the circularly permuted avidin is different from the wild type avidin biotin-binding affinity.

16. The circularly permuted avidin monomer of claim 1, characterized in that the HABA-binding affinity of the circularly permuted avidin is different from the wild type avidin HABA-binding affinity.

17. A dual-chain pseudo-tetrameric avidin, characterized in that it comprises two dual-chain avidins (dcAvd).

18. A dual-chain pseudo-tetrameric avidin of claim 17, characterized in that it binds biotin.

19. A single-chain avidin (scAvd), characterized in that it comprises two dual-chain avidin (dcAvd) molecules of claim 11 fused together to form a single polypeptide.

20. A single-chain avidin of claim 19, characterized in that the dcAvd-molecules are fused together via a linker.

21. A single-chain avidin of claim 20, characterized in that the linker is a 12 amino-acid linker GGSGSGSGSGSG.

22. An isolated polynucleotide encoding any of the avidin proteins of claims 1-21.

23. A recombinant vector comprising the polynucleotide of claim 22, wherein the polynucleotide is DNA.

24. A recombinant host cell comprising the polynucleotide of claim 22, wherein said polynucleotide is DNA.

25. A method for producing a polypeptide comprising expressing from the recombinant cell of claim 24 the polypeptide encoded by said polynucleotide.

AMENDED CLAIMS

[Received by the International Bureau on 21 April 2005 (21.04.2005): original claims 1-25 replaced by amended claims 1-22 (3 pages)]

Claims

1. A circularly permuted avidin monomer, where the carboxyl terminal amino acid and the amino terminal amino acid of the polypeptide of the avidin monomer have been joined directly or via a linker, and new carboxyl and amino termini have been created by cleavage of the polypeptide and the resulting circularly permuted avidin monomer binds biotin or other ligand, **characterized** in that the circularly permuted monomer is selected from the group comprising cpAvd5→4, cpAvd6→5, and cpAvd4→3.

2. The circularly permuted avidin monomer of claim 1, **characterized** in that the avidin is selected from wild type avidin, mutant form of avidin, streptavidin and variant of avidin, such as other poultry avidins and chicken avidin-related proteins (AVRs).

3. The circularly permuted avidin monomer of claim 1, **characterized** in that the carboxyl terminal amino acid and amino terminal amino acid have been joined by a linker comprising one or more amino acids.

4. The circularly permuted avidin monomer of claim 3, **characterized** in that the linker is a hexapeptide comprising four glycine residues and two serine residues and wherein one glycine is connected to the carboxyl terminal amino acid and one serine is connected to the amino terminal amino acid.

5. The circularly permuted avidin monomer of claim 1, **characterized** in that the biotin-binding affinity of the circularly permuted avidin is different from the wild type avidin biotin-binding affinity.

6. The circularly permuted avidin monomer of claim 1, **characterized** in that the HABA-binding affinity of the circularly permuted avidin is different from the wild type avidin HABA-binding affinity.

7. The circularly permuted avidin monomer of claim 1, **characterized** in that the monomer has been mutated.

8. The circularly permuted avidin monomer of claim 7, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to any other amino acid residue X and/or the isoleucine residue 117 to any other amino acid residue X and/or the serine residue 16 to any other amino

acid residue X and/or the threonine residue 35 to any other amino acid residue X and/or the asparagine residue 118 to any other amino acid residue X, (Y33X, I117X, S16X, T35X, N118X).

9. The circularly permuted avidin monomer of claim 8, **characterized** in that the monomer has been mutated by changing the tyrosine residue 33 to histidine residue and/or the isoleucine residue 117 to cysteine residue and/or the serine residue 16 to alanine residue and/or the threonine residue 35 to alanine residue and/or the asparagine residue 118 to methionine, (Y33H, I117C, S16A, T35A, N118M).

10. A dual-chain avidin (dcAvd), **characterized** in that it comprises a fusion of two of the monomers selected from the circularly permuted monomers of claim 1, cpAvd5→4, cpAvd6→5, and cpAvd4→3, or the mutated monomer forms of one of the claims 7 to 9, and the resulting dual-chain avidin binds biotin and/or other ligand.

11. A dual-chain avidin of claim 10, **characterized** in that the two monomers are fused together directly or joined by means of a spacer.

12. A dual-chain avidin of claim 11, **characterized** in that the spacer is a peptide spacer from about 1 to 40 amino acid residues.

13. A dual-chain avidin of claim 12, **characterized** in that the spacer is a peptide SGG or SGGS.

14. A dual-chain pseudo-tetrameric avidin, **characterized** in that it comprises two dual-chain avidins (dcAvd).

15. A dual-chain pseudo-tetrameric avidin of claim 14, **characterized** in that it binds biotin.

16. A single-chain avidin (scAvd), **characterized** in that it comprises two dual-chain avidin (dcAvd) molecules of claim 14 fused together to form a single polypeptide.

17. A single-chain avidin of claim 16, **characterized** in that the dcAvd-molecules are fused together via a linker.

18. A single-chain avidin of claim 17, **characterized** in that the linker is a 12 amino-acid linker GGSGSGSGSGSG.

19. An isolated polynucleotide encoding any of the avidin proteins of claims 1-18.

20. A recombinant vector comprising the polynucleotide of claim 19, wherein the polynucleotide is DNA.

21. A recombinant host cell comprising the polynucleotide of claim 19, wherein said polynucleotide is DNA.

22. A method for producing a polypeptide comprising expressing from the recombinant cell of claim 21 the polypeptide encoded by said polynucleotide.